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## The renal dopaminergic system in experimental hypertension. Renal hemodynamic studies in conscious rats.

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## INTRODUCTION

An elevated arterial pressure is an important health problem. There is a continuous relationship between the level of blood pressure and the risk of cardiovascular and renal target organ damage.<sup>1,2</sup> In most patients with arterial hypertension no circumscribed cause is present. These patients are said to have essential hypertension. The primary difficulty in finding the mechanism responsible for essential hypertension is attributed to the variety of factors that are involved. There is evidence that both environmental (salt intake, obesity, occupation etc) and genetic factors contribute to the development of hypertension.

Several abnormalities have been described in essential hypertension that may be primarily responsible for the hypertension. In particular, there is evidence for a causal role of the kidney in essential hypertension. Renal transplantation studies in animal models and humans demonstrate that "hypertension follows the kidney", i.e. implanting a kidney of a hypertensive subject in a normotensive subject will induce hypertension and vice versa.<sup>3</sup> The kidney plays a central role in the long-term regulation of blood pressure and of sodium and volume homeostasis. This is accomplished by an intricate interaction between multiple factors, which affect renal sodium excretion and thereby regulate the effective circulating volume. These factors may have a direct effect on sodium excretion by acting at a tubular level and inhibiting sodium reabsorption or indirectly by acting on renal hemodynamics.

In the 1970's it was recognized that dopamine, an intrarenally produced hormone, is also an important regulator of renal function. It became apparent that dopamine has hemodynamic as well as tubular effects. The hemodynamic effects include renal vasodilatation with a slight rise in glomerular filtration rate. The tubular effects entail natriuresis and diuresis. Dopamine regulates renal function by stimulation of specific renal dopaminergic receptors which are classified in two groups, i.e., D<sub>1</sub>-like and D<sub>2</sub>-like receptors.

Experimental and clinical studies suggest that defects in the renal dopaminergic system, resulting in an impaired sodium and volume excretion, may play a role in the pathogenesis of essential hypertension.<sup>4</sup> In patients with essential hypertension a deficiency in renal dopamine synthesis and/or secretion has been reported.<sup>5,6</sup> In

the Spontaneously Hypertensive Rat (SHR), a defect in the renal dopaminergic system, resulting in an impaired sodium handling, has been demonstrated. It is unclear whether the defect is due to a deficiency in D<sub>1</sub>-like receptors or whether the receptors are present but the receptor agonist is present in lower concentrations.

The present thesis describes the hemodynamic responses to a D<sub>1</sub>-like receptor agonist in the SHR. The function of the dopaminergic system (RAAS) may be studied by studying the renal response to sodium intake thereby regulating the effective circulating volume. Second, we study the effect of pharmacological blockade of the dopaminergic system on the renal response to sodium intake.

## Chapter 1

To explore the effect of the dopaminergic system on the renal response to sodium intake in the SHR, we first performed a series of experiments in the SHR. In small experimental animals, the renal response to sodium intake is often performed under conditions of catheterization, as these animals are small. However, catheterization can give rise to several drawbacks we established in the SHR. The renal response to sodium intake in unrestrained spontaneously hypertensive rats (SHR) was measured as the urinary excretion of sodium. The renal response to sodium intake for incomplete bladder obstruction was measured as the urinary excretion of sodium.

<sup>125</sup>I-iothalamate was used to measure the renal plasma flow (RPF) in the SHR.

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the Spontaneously Hypertensive Rat (SHR), an animal model of essential hypertension, a defect in the tubular  $D_1$ -like receptors has been associated with impaired sodium handling.<sup>7</sup> However, it is unclear whether the renal hemodynamic response to a  $D_1$ -like receptor agonist is also impaired in SHRs. In addition, it is unclear whether the defects in the dopaminergic system are limited to  $D_1$ -like receptors or whether also a defect in the renal vascular response to a  $D_2$ -like receptor agonist is present.

The present thesis aims to elucidate whether in SHRs the renal hemodynamic responses to a  $D_1$ -like and  $D_2$ -like agonist are impaired. In vivo, the renal hemodynamic response to dopaminergic stimulation may depend not only on the function of the dopaminergic cascade as such, but also on interaction with other neurohormonal systems. The interaction with the renin angiotensin aldosterone system (RAAS) may be particularly relevant as renal vasoconstrictor responses to angiotensin II are enhanced in SHRs. We investigated the impact of this interaction by studying the renal effects of dopaminergic stimulation during low and high sodium intake thereby modifying the extent of RAAS activation in a physiological way. Second, we studied the renal effects of dopaminergic stimulation during pharmacological blockade of the AT1-receptor.

### Chapter 1

To explore the effect of selective dopaminergic stimulation on renal hemodynamics in the SHR, we first had to develop a model for measuring renal function in rats. In small experimental animals renal function measurements by clearance methods are often performed under anesthesia or with the application of restrainers and bladder catheters, as these measurements rely on accurately timed and collected urine samples. However, anesthesia and stress can affect renal function and bladder catheters can give urinary tract infections, and bladder stones.<sup>8</sup> To avoid these drawbacks we established a model for measuring renal function in conscious unrestrained spontaneous voiding rats. Glomerular Filtration Rate (GFR) was measured as the urinary clearance of constantly infused  $^{125}\text{I}$ -iothalamate. To correct for incomplete bladder emptying, a well-known problem in rats, urinary clearance of  $^{125}\text{I}$ -iothalamate was multiplied by the ratio of plasma and urinary clearance of

simultaneously infused  $^{131}\text{I}$ -hippuran. This correction method has previously been validated in man.<sup>9</sup>

In chapter 1 we tested the feasibility of this renal function measurement technique in rats with normal and moderately impaired renal function induced by adriamycin. The intra-assay variation of the technique was evaluated by analysis of the results of 4 consecutive clearance periods during the day. Furthermore we evaluated the inter-assay variation by comparing clearance periods on separate days in another group of rats. Application of the correction for voiding errors significantly reduced the intra-assay coefficient of variation of GFR from  $27.4 \pm 14.3$  to  $5.3 \pm 2.3$  % in rats with normal renal function and from  $27.9 \pm 20.7$  to  $2.7 \pm 1.6$  % in rats with moderately impaired renal function. The inter-assay coefficient of variation fell, albeit not statistically significant (from  $23.4 \pm 10.3$  to  $11.0 \pm 7.2$  %). Our data show that this correction method is a useful technique to assess renal function in conscious, spontaneously voiding rats.

## Chapter 2

A disadvantage of our method to measure GFR in conscious spontaneously voiding rats is the requirement of an intra-arterial catheter for infusion of the renal function tracers. Preparation of animals with chronic arterial catheters is difficult and may increase mortality. Furthermore there is a high occlusion rate. We therefore tested whether intraperitoneal infusion of  $^{125}\text{I}$ -iothalamate and  $^{131}\text{I}$ -Hippuran can be used for GFR measurement in conscious spontaneously voiding rats. We found that during intraperitoneal administration stable plasma levels of  $^{131}\text{I}$ -Hippuran could be obtained. However, urinary recovery of  $^{131}\text{I}$ -Hippuran was incomplete ( $66 \pm 32$  %), leading to a significant overestimation of GFR by  $140 \pm 13$  % in comparison with GFR measured by the intra-arterial technique. Thus, unfortunately the intraperitoneal route of continuous infusion of renal function tracers cannot replace intra-arterial infusion.

## Chapter 3

We applied our method of renal function measurement to elucidate whether, in line with the previously described impaired renal tubular dopaminergic responses, the

renal hemodynamic SHR under normal p renal hemodynamic which may be due groups.<sup>10,11</sup> In chapter to the continuously normotensive Wistar control the effects of observed effects of studied the animals c

We found that fe plasma flow (ERPF) SHRs ( $+7 \pm 3$  %, significantly lowered systemic vasodilator reduction in renal va 2 %) than in SHRs was not due to imp ACE inhibitor - indu blunting of the renal sodium intake, as it the renal hemodyn conscious SHRs are vasodilator response and was not due to support the present pathway in SHRs.

## Chapter 4

Dopamine, the end exact role of periph not clear. To date a

renal hemodynamic responses to a  $D_1$ -like and  $D_2$ -like agonist are impaired in the SHR under normal physiological conditions. To date the few studies on the topic of renal hemodynamic  $D_1$ -like responsiveness in SHRs provide conflicting results, which may be due to the use of anesthesia or due to the absence of control groups.<sup>10,11</sup> In chapter 3 we therefore compared the renal hemodynamic responses to the continuously infused  $D_1$ -like agonist fenoldopam in conscious SHR and in normotensive Wistar Kyoto rats (WKY). In addition, we measured as an active control the effects of the ACE inhibitor captopril. To exclude the possibility that the observed effects of fenoldopam were related to the prevalent sodium status, we studied the animals on a low as well as a high sodium diet.

We found that fenoldopam ( $2 \mu\text{g/kg.min}$ ) significantly increased effective renal plasma flow (ERPF) in WKY rats ( $+22 \pm 5 \%$ ) whereas this response was absent in SHRs ( $+7 \pm 3 \%$ , NS). Despite the absent increase in ERPF, fenoldopam significantly lowered mean arterial pressure (MAP) in SHRs demonstrating a systemic vasodilator response to fenoldopam in this strain. As a consequence, the reduction in renal vascular resistance (RVR) was more pronounced in WKYs ( $-24 \pm 2 \%$ ) than in SHRs ( $-13 \pm 4 \%$ ) ( $p < 0.05$ ). The impaired renal vasodilator response was not due to impaired vasodilator capacity, as demonstrated by a significantly ACE inhibitor - induced increase in ERPF in SHRs ( $+16 \pm 3 \%$ ,  $p < 0.001$ ). The blunting of the renal vasodilator response to fenoldopam in SHRs is independent of sodium intake, as it was present during a high as well as a low sodium diet. Thus, the renal hemodynamic responses to the  $D_1$ -like receptor agonist fenoldopam in conscious SHRs are blunted under physiological conditions. The difference in renal vasodilator response between SHR and WKY rats did not depend on sodium intake and was not due to an impaired renal vasodilator capacity. Therefore, our findings support the presence of a functional defect in the renal vascular dopaminergic pathway in SHRs.

#### Chapter 4

Dopamine, the endogenous ligand, acts on both  $D_1$ -like and  $D_2$ -like receptors. The exact role of peripheral  $D_2$ -like receptors in the regulation of renal hemodynamics is not clear. To date an increase as well as a decrease in ERPF and GFR in response

to D<sub>2</sub>-like stimulation has been described.<sup>12-14</sup> These discrepancies may be due to the use of anesthesia, different D<sub>2</sub>-like agonists, and differences in route of administration and differences in sodium status. We were especially interested whether, similarly as the D<sub>1</sub>-like renal vascular response, the renal vascular response to a D<sub>2</sub>-like receptor agonist is impaired in SHR. To determine renal hemodynamic responsiveness to D<sub>2</sub>-like stimulation in SHR we studied the effect of a continuous infusion of the D<sub>2</sub>-like agonist N,N-Di-n-propyldopamine (DPDA) (10 µg/kg.min) on systemic and renal hemodynamics in conscious SHR under low sodium (LS) and high sodium (HS) conditions as compared to the normotensive WKY strain. The specificity of DPDA was tested by co-administration of the D<sub>2</sub>-like receptor antagonist domperidone. DPDA slightly reduced MAP in SHR and in WKYs under LS and HS conditions. ERPF decreased to a similar extent in SHR (LS -20 ± 4 %, HS -20 ± 2 %) and WKYs (LS -14 ± 2 %, HS -17 ± 2 %). As a consequence, RVR increased significantly in both strains. The renal hemodynamic response to DPDA was absent during pre-treatment with domperidone, indicating the specificity of DPDA for the D<sub>2</sub>-like receptors.

In conclusion, our data show a preserved D<sub>2</sub>-mediated renal vasoconstriction in SHR. Thus, during endogenous dopaminergic stimulation, the impaired D<sub>1</sub>-mediated renal vasodilator along with the preserved D<sub>2</sub>-mediated renal vasoconstrictor response may result in renal vasoconstriction rather than vasodilatation.

## Chapter 5

The mechanism of the impaired renal vasodilator response to a D<sub>1</sub>-like agonist, as described in chapter 3, is unclear. The impaired response may be due to an uncoupling of the D<sub>1</sub>-like receptor from the Gs-protein adenylyl cyclase enzyme complex (its effector enzyme complex), a similar mechanism has been reported for the renal tubular defect.<sup>15</sup> On the other hand the blunted renal vasodilator action to D<sub>1</sub>-like stimuli may be caused by an altered balance between the dopaminergic system and renin angiotensin aldosterone system (RAAS). It is well known that D<sub>1</sub>-like stimulation induces renin release.<sup>16,17</sup> Activation of the RAAS by D<sub>1</sub>-like stimulation may lead to an impaired renal vascular D<sub>1</sub>-like responsiveness in SHR

as there is evidence more enhanced in S renal hemodynamic after chronic blockade

Fenoldopam reduced accordance with our more in WKYs (+26 receptor antagonist both strains. Pre-treatment increase in ERPF to treatment with an / similar in both strain. Thus, under physiological blunted renal vasodilation implies that the blunted defect, or an impaired defective interaction

## Implications and future

Physiological, biochemical endogenous dopamine and body volume homeostasis the dopaminergic system with the previously tubules we found agonist in SHR. The systemic vascular D<sub>1</sub>-like stimulation limited to D<sub>1</sub>-like receptors to a D<sub>2</sub>-like receptors

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as there is evidence that the renal vasoconstrictor response to angiotensin II is more enhanced in SHR than in WKY rats.<sup>18</sup> Therefore we were interested in the renal hemodynamic responses to a D<sub>1</sub>-like receptor agonist in SHRs before and after chronic blockade of the RAAS by an AT<sub>1</sub> receptor antagonist.

Fenoldopam reduced MAP to a comparable degree in WKYs and in SHRs. In accordance with our previous findings, fenoldopam increased ERPF significantly more in WKYs (+26 ± 2 %) than in SHRs (+2 ± 2 %). Treatment with an AT<sub>1</sub> receptor antagonist reduced MAP and increased ERPF and GFR significantly in both strains. Pre-treatment with an AT<sub>1</sub> receptor antagonist induced a significant increase in ERPF to fenoldopam in SHRs, but not in WKYs. As a result, during pre-treatment with an AT<sub>1</sub> receptor antagonist, the rise in ERPF by fenoldopam was similar in both strains (SHR +25 ± 2 %,  $p < 0.0001$  / WKY +33 ± 2 %,  $p < 0.0001$ ). Thus, under physiological conditions, blockade of RAAS activity normalizes the blunted renal vasodilator response to a D<sub>1</sub>-like agonist in conscious SHRs. This implies that the blunted vasodilator response is not due to a structural receptor defect, or an impaired vasodilator capacity of renal vascular bed in SHRs, but to a defective interaction between the dopaminergic system and the RAAS.

#### Implications and future perspectives

Physiological, biochemical and molecular studies show the importance of endogenous dopamine and renal dopamine receptors in the regulation of sodium and body volume homeostasis. Several lines of evidence suggest that a defect in the dopaminergic system is involved in the pathophysiology of hypertension. In line with the previously described D<sub>1</sub>-like receptor coupling defect in the proximal tubules we found an impaired renal vasodilator response to a D<sub>1</sub>-like receptor agonist in SHRs. This defect is present in the renal vascular bed and not in the systemic vascular bed as we found a normal systemic vasodilator response during D<sub>1</sub>-like stimulation. In addition, this defect in the dopaminergic system seems to be limited to D<sub>1</sub>-like receptors as we observed that the renal vasoconstrictor response to a D<sub>2</sub>-like receptor agonist is preserved in SHRs.

Dopamine, an intrarenal hormone, activates both dopaminergic receptor types simultaneously and is mainly released under conditions of intravascular volume